

REMARKS

Rejections Under 35 U.S.C. § 103(a)

Claims 25-27, 32-36, 38, and 42 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Li *et al.* (Brain Research Protocols, 2000, 5: 21 1-21 7), in view of both Lopez Garcia *et al.* (Analyst, 1991, 11 6: 51 7-520) and Stemmer *et al.* (US Patent No. 5,834,252).

The Examiner alleges that the Li reference teaches real time PCR, but fails to teach the use of antifoam agents. To fill the deficiencies in Li *et al.*, the Examiner cites Lopez Garcia *et al.* and Stemmer *et al.* The Examiner alleges that Lopez Garcia *et al.* teaches "that small bubbles formed in the presence of detergents worsen the reproducibility of quantification by optical detection" and "using anti-foaming agents to over come this problem" Office Action of June 5, 2009, at page 3. The Examiner also alleges that Stemmer teaches that antifoam-agents could be used in PCR, and concludes from the combination of the references that it would be obvious to employ an anti-foam agent in real time PCR to improve the accuracy of optical detection. Applicants respectfully traverse.

Applicants reassert and incorporate their arguments of record regarding the teachings of Stemmer.

In citing Stemmer *et al.* and Lopez Garcia the Examiner picks and chooses from the disclosures only so much as to support her obviousness rejection. This type of "cherry picking" is improper as the Federal Circuit has stated:

[i]t is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art. (*Bausch & Lomb, Inc., v. Barnes-Hind/Hydrocurve, Inc.*, 796, F.2d 443, 448 (Fed. Cir. 1986) (quoting *In re Wesslau*, 355 F. 2d 238, 241 (CCPA 1965)).

Nothing in Stemmer teaches or describes quantitative PCR, as recited in the instant claims. Moreover, Stemmer must be considered for all that it teaches, not merely any part of the reference that supports the Examiner's assertions regarding obviousness. In particular, as previously described in the Berninger declaration, Stemmer suggests use of conditions that are incompatible with either PCR or quantitative PCR. One of ordinary skill in the art reading

Stemmer clearly would recognize this incompatibility and would not be motivated by any alleged teachings regarding PCR.

Similarly, nothing in the Lopez Garcia reference can be said to describe an issue with small bubbles formed in the presence of detergents worsening the reproducibility of quantification by optical detection, when the entirety of the reference is considered. That reference states:

When surfactant concentration was higher than about 0.2%, the slurries gave rise to abundant foam and small air bubbles were trapped in the sample loop of the FI manifold, making reproducibility worse.

Applicants respectfully submit that the FI manifold, which is described in the Experimental section on page 517 of Lopez Garcia, is upstream of the optical path. Put more simply, Lopez Garcia appears to be discussing, for want of a better term an "air lock" or partial "air lock" in the tube that feeds the nebulizer of the atomic absorption instrument. Applicants respectfully submit that bubbles at that point in the sample path simply are not present in the optical path. Even if air bubbles passed through the nebulizer they would be destroyed in the high temperatures present in the flame of the instrument prior to reaching the optical path. In sum, the optical path passes through gas phase materials (e.g., the flame and/or its effluent), not liquids where air bubbles could exist. In view of the foregoing, Applicants respectfully submit that after a complete reading, a skilled artisan would understand that Lopez Garcia does not teach, and indeed cannot be cited for teaching, air bubbles interfere with optical detection due to their presence in an instrument's optical path, or that antifoam agents affect that problem.

Lopez Garcia, which is directed to atomic absorbance spectroscopic techniques, cannot be considered analogous art capable of being combined with references directed to PCR. Atomic absorbance spectroscopy as applied in that reference requires the destructive testing liquid sample by drawing them into a flame, which precludes the repeated sample analysis over multiple cycles that is necessary for real time PCR. The reference deals with the analysis of metal particle slurries and the difficulties of foaming in those samples, not in compositions used for polymerase chain reaction. Moreover, nothing in Lopez Garcia teaches or fairly suggests that nucleic acids, or for that matter any other non-metal containing organic molecule, can be quantitated by atomic absorption spectrometry as such molecules would be burned in the

process. For at least the foregoing reasons, Lopez Garcia cannot be said to be analogous art, or event to stand for the proposition relied upon by the Examiner in formulating the rejection.

Further to the foregoing, Applicants submit that at the time of their invention, it would have been considered inconsistent with the art-recognized stabilizing effect of detergents on purified polymerase enzymes to employ a polymerase in an assay/reaction mixture containing an antifoam agent as antifoam agents interact with detergents and can render them unavailable to stabilize the polymerase. This is evidenced by the teachings of U.S. Patents 6,127,155 and 6,242,235, and EP1970440A1, which are submitted herewith and that will be discussed in a declaration by Dr. Michael Smith that will be submitted hereafter.

In view of the preceding remarks, Applicants respectfully submit that a *prime facie* case of obviousness has not been established as the Lopez Garcia and Stemmer references fail to remedy the admitted deficiencies of the Li *et al.* Moreover, the references as set forth above do not provide a motivation for their combination, particularly as the Lopez Garcia reference does not teach problems with bubbles present during optical detection as alleged by the Examiner.

In the event that the Examiner seeks to maintain that a *prima facie* case of obviousness exists despite the manifold deficiencies of the cited reference, Applicants submit that any evidence of obviousness is rebutted by the surprising results described in Applicants' specification. Specifically, Applicants' specification shows that the instantly claimed methods surprisingly allow detection measurements that are free of artifacts. For example, FIGS 2 and 4 of the instant specification illustrate the deleterious effect of foaming on threshold cycle (Ct) determination in real-time PCR from identical reactions containing 20 copies of template DNA per reaction. The only difference between the two reactions is the inclusion of antifoam in the reactions recorded in FIG 4. FIG 2 shows that fluorescence readings were distorted with respect to baseline and Ct determinations in qPCR reactions without the addition of anti-foaming agents. Specifically, the bubble error in wells H1 to H6 resulted in Ct values ranging from 33 to 38, which represents approximately a 15 fold difference. FIG 4, however, surprisingly shows that the addition of anti-foam not only limited bubble formation, but produced a stable baseline allowing for a more accurate reading. The greatest variation in these antifoam containing tests was 1 Ct, which represent approximately a 2 fold difference in quantification.

In summary, Applicants respectfully submit that the Examiner has failed to present a *prima facie* case of obviousness. Moreover, even if, for the sake of argument only, it is assumed

that a *prima facie* case of obviousness exists, the evidence of surprising results as described above, negates such *prima facie* case. Accordingly, withdrawal of the rejection respectfully is requested.

Claims 25-28, 32-38, and 42 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Li *et al.* taken with both Lopez Garcia *et al.* and Stemmer *et al.*, in further view of Blaschke *et al.* (J Immunol Methods, 2000, 246: 79-90).

Claims 25-27, 29-36, and 38-42 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Li *et al.* taken with both Lopez Garcia *et al.* and Stemmer *et al.*, in further view of each Kyle (US Patent No. 5,658,767), Sigma catalog (1998) and Wierenga (US Patent No. 5,968,889).

Applicants traverse the two secondary rejections adding either Blaschke, or the combination of Kyle (US Patent No. 5,658,767), the Sigma Catalog and Wierenga (US Patent No. 5,968,889) to the primary combination of Li in view of Stemmer and Lopez Garcia. Neither Blaschke, nor the combination of Kyle, the Sigma Catalog and Wierenga cure the deficiencies of Li, Stemmer and Lopez Garcia. For at least those reasons, Applicants respectfully submit that *prima facie* cases of obviousness have not been presented in those rejections.

In view of the foregoing remarks, Applicants respectfully submit that the application is in condition for allowance. Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact the undersigned to expedite prosecution of the application.

The Commissioner is hereby authorized by this paper to charge any fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-2283. **This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).**

Respectfully submitted,

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Perkins Coie LLP

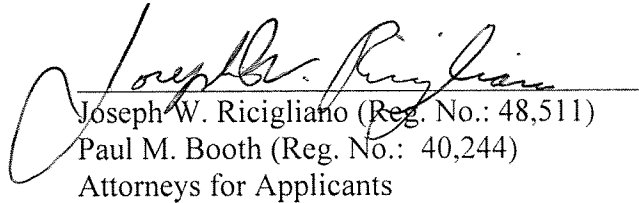
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